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13. ABSTRACT *(Maximum 200 words)*

Variations in interbeat interval (IBI) determined from the ECG of telemetry-equipped rats were evaluated as markers of physiological status during hypothermia and re-warming. Animals were cooled to a core temperature (Tc) of 19-20oC, maintained there for 30 min, and then re-warmed. Out of 19 hypothermia inductions, 14 were successfully re-warmed (S), and 5 were unsuccessfully re-warmed (US). ECG and Tc data were collected for 10 sec every 5 min. Six 10-sec strips were combined into blocks: Block 1- start of cool, Block 2- midway through cooling, Block 3- coldest period, and Block 4- end of rewarming. Lorenz plots of IBI's in Blocks 1-4 demonstrated markedly increased IBI values with coldest Tc's in Block 3; US exhibited greater scatter in Block 3 than S. NSD (normalized standard deviation) of Block 1 was lower ($p < 0.05$) than any other Block for both S and US trials. NSD's in US trials were higher ($p < 0.005$) than for S for Blocks 1-3. Other time domain measures (SDSD and RMSSD) showed significant differences between S and US rats in Block 3 only. This study in time domain analyses indicates that NSD may be the most useful in predicting fatal hypothermia when compared to RMSSD and SDSD.

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BIOPHYSICAL FACTORS INHERENT IN HEART RATE VARIABILITY OF RATS DURING COOLING AND RE-WARMING

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EXECUTIVE SUMMARY

Variations in inter-beat cardiac frequency intervals (IBI) determined from the ECG of telemetry-equipped rats were evaluated as markers of physiological status during hypothermia and re-warming. ECG and core temperature (Tc) were measured in telemetry equipped male rats (N=14). After 2 weeks of recovery from implantation surgery, rats were anesthetized and placed into coils of copper tubing through which temperature-controlled water was circulated. Animals were cooled to a Tc of 19 to 20°C, maintained there for 30 minutes, and then re-warmed. Out of 19 hypothermia inductions (5 of the 14 rats were subjected to hypothermia twice), 14 inductions were successful (S). Five rats were unsuccessfully cooled and re-warmed (US). ECG data were collected for 10 seconds every 5 minutes. The six 10 sec. strips were combined into blocks to represent the following 30 min. periods: Block 1- start of cooling (Tc=34.3 ± 3.0°C), Block 2- midway through cooling (Tc= 24.8 ± 2.1°C), Block 3- coldest period (Tc =19.8 ± 0.7°C), and Block 4- end of re-warming (Tc=32.7 ± 1.8°C). The R-R intervals of the ECG waveforms were marked and IBI's were determined for each Block. Lorenz plots of these data in Blocks 1-4 demonstrated markedly increased IBI values with coldest core temperatures in Block 3; US exhibited greater scatter in Block 3 than S. Normalized standard deviations (NSD) of S trials indicated that Block 1 was significantly ($p<0.05$) lower than Block 2, 3 and 4. NSD of US trials indicated that Block 1 was significantly lower than Blocks 2 and 3. Also, NSD in US trials were significantly ($p<0.005$) higher than for S trials for Blocks 1, 2 and 3. Standard Deviation of Successive Differences (SDSD) and Root Mean Square of Successive Differences (RMSSD) showed significant differences between S and US rats in Block 3. This study in time domain analyses for HRV indicates that NSD of IBI may be the most useful in predicting fatal hypothermia when compared to other time domain analyses. Such biophysical techniques for analysis of IBI may be applied for downstream time domain algorithms used in human data sets.

INTRODUCTION

Heart Rate Variability (HRV) is defined as the variation of R to R intervals, or inter-beat intervals (IBI), between consecutive QRS complexes in ECG waveforms. Decreases in HRV have been used to detect fetal distress and heart damage in patients with myocardial infarctions (Axselrod, 1981, Malik *et al.*, 1996). Three physiological events that are responsible for most of HRV are changes in blood pressure, respiration, and thermoregulatory effects that involve the modulation of peripheral blood flow (Hyndman, 1971).

This study is in support of the Warfighter Physiological Status Monitoring (WPSM, STO H) program. The experiments, in a non-human species, were designed to gather appropriate complex wave pattern technologies that potentially track and measure physiological human status over wide thermoregulatory compromised circumstances.

In humans, mild hypothermia occurs when Tc drops to 34-36°C with increases in heart rate, blood pressure and cardiac output. Moderate hypothermia (Tc=30-34°C) includes decreases in heart rate, blood pressure and cardiac output and the appearance of cardiac arrhythmias. Severe hypothermia (Tc<30°C) symptoms are similar to moderate hypothermia except that they become more severe. (Weinberg, 1993)

IBI data from humans and rats can be analyzed in many ways including frequency domain and time domain analyses. Frequency domain analysis tends to be more complex than time domain. Frequency domain analysis or spectral analysis generates a cumulative power spectrum of a series of IBI's in multiple intervals. Power is defined as the square of variance (Kleiger *et al.*, 1995). Changes in different peaks in the PSD are correlated with changing physiology (Sayers, 1973). Frequency domain analysis studies in humans and rats have found trends within certain regions of the PSD. Studies (Nelskyla *et al.*, 1999, Cerutti, *et al.* 1991, Perlini, *et al.*, 1995) have determined that changes in low (very low in human data) frequency are due to, primarily, sympathetic stimulation from the thermoregulatory effects of the modulation of blood flow. Mid (Low in human data) frequency is a combination of sympathetic and parasympathetic stimulation from blood pressure and high frequency is mostly parasympathetic stimulation from respiration.

Time domain analyses are more direct than frequency domain analyses. Time domain analyses are calculated using IBI's or the differences between successive IBI's. For this study, we used comparisons of the mean, standard deviation (SD), normalized standard deviation (NSD), standard deviation of successive differences (SDSD), root mean square of successive differences (RMSSD) and Lorenz plots of IBI's in different blocks to detect changing physiology. SD is an estimate of total power or the total variability (Kleiger, 1995) of the IBI's within a Block. Since SD values increase with larger IBI values, which are experienced at low Tc's, it was determined that a more accurate measure of total variability would be a normalized value of SD (NSD).

Therefore NSD would not be influenced by increases or decreases in heart rate (HR). The Lorenz plot is a geometric representation of the dispersion or chaos of IBI's within a block. SDSD and RMSSD are based on the successive differences of IBI's. SDSD and RMSSD are measures that estimate the variability that correlates to the high frequency region of a PSD curve for short-term sampling of waveforms (Malik, *et al.* 1996).

In this study, IBI's from the ECG waveforms from hypothermic rats were examined to determine if signatures could be developed to detect and predict physiological stress leading to hypothermia mortality. These signatures could prove to be valuable in predictive modeling of human responses.

METHODS

Animals and Housing: All experimental procedures were approved by our Institutional Animal Care and Use Committee and carried out with adherence to the "Guide for the Care and Use of Laboratory Animals," as revised in 1996 and to the U.S. Government Principles for Animal Use, 1985. Fourteen male Harlan Sprague-Dawley rats were housed in the animal colony in accordance with the American Association for the Accreditation of Laboratory Care standards. Animals were independently housed in wire bottom cages in environmentally controlled conditions (26°C, 50% relative humidity). Automatic lighting was used (on: 0600-1800) and food (Purina rat chow) and water were available *ad libitum*

Telemetry: Each animal had a telemetry device (Data Sciences, St. Paul, Minnesota, USA, TL11M2-C50-PXT) surgically implanted. These transmitters measured activity, core temperature, blood pressure (systolic and diastolic), heart rate, and ECG waveforms. Surgical implantation was accomplished under pentobarbital anesthesia (Nembutal, 45 mg/kg intraperitoneal; atropine, 200 ug intramuscularly; Polyflex® ampicillin, 12.5 mg intramuscularly) using aseptic technique 2 weeks prior to experimentation. As previously described (Matthew, 1997), the blood pressure catheter was inserted and fixed non-occlusively into the abdominal aorta between the renal artery and the iliac bifurcation, and the ECG leads were tunneled subcutaneously and fixed to the chest wall musculature in the Lead II configuration. BP and ECG waveforms are reported separately in Matthew, under review.

Hypothermia/re-warming: Rats were lightly anesthetized (Nembutal, 35mg/kg i.p.) and placed into coils of copper tubing thru which temperature controlled water was circulated. Animals were cooled to a Tc of 19-20°C and maintained at that temperature for 30 minutes. Animals were then re-warmed to a Tc of 35 °C. As the animals were cooled and then re-warmed, ECG and Tc data was recorded for 10 seconds every five minutes. Due to updates in technology some rats' data was taken continuously, however, the data used was 10 seconds every five minutes. The rats were then returned to their cages to recover. There were a total of 19 hypothermia inductions in 14 animals. Five animals were retested 4 to 29 days after the first induction. One rat

was successfully cooled and re-warmed the first time but not the second time. This gives a total of 14 S inductions and 5 US inductions.

Data Analysis: The ECG waveform data was marked to determine the inter-beat intervals (IBI). Six consecutive ten second pieces (30 minutes) were then combined to form a Block. Block 1 was the start of cooling with mean Tc of all 19 inductions of 34.3 ± 3.0°C, Block 2 was midway through cooling with mean Tc of 23.4±1.0°C, Block 3 was the coldest period with mean Tc of 19.5±0.5°C and Block 4 was the end of re-warming with mean Tc's, for S inductions only, of 32.8±1.8°C. Figure 1 in Appendix A demonstrates the location of the data that was taken to form Blocks 1-4 on a representative cooling curve.

The IBI's for Blocks 1-4 were plotted N vs. N-1 to form Lorenz or scatter plots in Figures 2-11, Appendix A. These Lorenz plots demonstrated the dispersion of IBI's in each block for each rat.

Mean IBI and NSD were also plotted in Figure 12, Appendix A. NSD was calculated by the following:

$$NSD = \frac{\text{SD of IBI}}{\text{Mean IBI}}$$

Where SD is the standard deviation of the IBI's in a Block divided by the mean of the IBI's in the same block.

Another time domain analysis used was the root mean square of successive differences, RMSSD. The following formula, as described by Kleiger *et al.*, 1995, was used:

$$RMSSD = \sqrt{\left(\frac{\sum (X_n - X_{n+1})^2}{N} \right)}$$

Where the successive difference is $(X_n - X_{n+1})$ and N equals the number of IBI's used.

The standard deviation of successive differences (SDSD) was also used. The formula for SDSD, also described by Kleiger *et al.*, 1995, is as follows:

$$SDSD = \sqrt{\left(\frac{\sum (\mu - X_n)^2}{n-1} \right)}$$

Where Xn equals the successive difference and μ equals the mean of all successive differences and n equals the number or count of differences.

Statistical Analysis: Repeated Measures ANOVAs were used to determine whether mean IBI's and NSD's were significantly different among Blocks 1, 2, 3 and 4. "t"-tests were used to determine significant differences between S and US groups in each Block for IBI, NSD, SDSD and RMSSD. "t"-tests were also used to determine if there were differences between first and second inductions of hypothermia and re-warming.

RESULTS

The first time domain measure used in this study was the Lorenz plot. Figures 2-11 are Lorenz plots of IBI N vs. N-1. These figures demonstrate the dispersion of points or chaos for each Block of successfully (S) and unsuccessfully (US) cooled and re-warmed rats. Markedly increased IBI values occurred with the coldest Tc in Block 3 in S. US trials demonstrate even greater scatter in all blocks but most noticeably in Block 3 than S trials. Figure 8B represents the first hypothermia induction and successful re-warming of Rat 99063. Figure 9B represents the second hypothermia induction from which 99063 was not successfully re-warmed. Note increased chaos in all blocks in 9B compared to 8B.

In Figure 12 the mean IBI and NSD of S and US trials were plotted. The mean IBI's for S rats demonstrate that Block 1 is different ($p<0.05$) from Blocks 2 and 3. Block 4 is different from Blocks 2 and 3. For US rats, Block 1 is different from Block 3. Mean IBI demonstrate significant differences between S and US rats in Blocks 1 and 2. The NSD of Block 1 for S rats is significantly lower than for NSD in Blocks 2, 3 and 4. For US rats, Block 3 is different from Blocks 1 and 2. The NSD of Blocks 1, 2 and 3 for US trials is significantly ($p<0.005$) higher than for S trials.

In Table 1, Appendix B, the mean IBI, NSD, RMSSD and SDSD values for S and US rats in each Block are presented. This table summarizes the significances between S and US rats in each Block for each time domain measure. For mean IBI, US is different from S rats in Blocks 1 and 2. NSD demonstrates differences between S and US rats in Blocks 1, 2 and 3. For RMSSD and SDSD, time domain estimates of HF power (Kleiger *et al.*, 1995), a significant difference between S and US rats was discovered only in Block 3.

DISCUSSION

The advantages of time domain analyses are that they are relatively easy to calculate and provide optimum estimates of total variability for short-term and long-term sampling of IBI data in data with substantial stationarity. Frequency domain analysis is more powerful and descriptive in determining the cause of variability by the separation into different frequency ranges. However, frequency domain analysis is dependent on continuous ECG waveforms. The data presented in this report is not continuous data, therefore analysis was limited to time domain analyses.

The IBI data in hypothermic rats shows the predicted increase in time between R to R intervals as Tc decreases (Figures 2-12). The Lorenz plots (Figures 2-12) demonstrate the chaos in the distribution of IBI's at different Tc's. There are many methods in measuring this chaos such as the HRV triangular index measurement. (Malik *et al.*, 1996) In this study, we chose the normalized standard deviation. This value would not be affected by increases or decreases in heart rate and would provide a value that represents the total variability of the IBI's in each Block. With a decrease in

heart rate, IBI increased and NSD increased. Some of this variability may stem from an increased probability of ectopic beats occurring in low Tc's (Soloman, 1989). Ectopic beats are defined as beats that occur from a site other than the Sino-Atrial (SA) node. Other studies (Perlini, *et al.*, 1995, Fleisher, *et al.*,1996) have edited or excluded ectopic beats in their analysis of HRV. In the Lead II configuration used for the placement of the ECG electrodes in the rat, distinguishing the smaller ECG waves such as P and T from baseline noise is difficult. This further complicates identification of normal and ectopic beats. In this study, all R to R intervals were marked to decrease the probability of over-editing the waveform. So the increase in ectopic beats could contribute in the increased NSD seen, especially in the US rats.

When RMSSD and SDSD were calculated, S rats were significantly lower than US rats in Block 3. The increased RMSSD and SDSD values may result from sporadic breathing that can occur in very low Tc's. RMSSD and SDSD are time domain measures that correlate to the HF range in frequency domain analysis (Malik *et al.*, 1996). The HF range represents the variability that occurs mainly from respiration (Sayers, 1973). The unsuccessfully recovered rats appeared to be the result of respiratory arrest. Wong, 1983 and Kiley *et al.*, 1984 have noted that hypothermia affects respiratory drive and timing in animals near a Tc of 20°C. This cold Tc cools the respiratory center causing decreased ventilation. In a previous study (Matthew, 2002) using the same methods for cooling and re-warming, out of 120 rats no fatalities from cooling and re-warming occurred. During that experiment, when animals appeared to be in respiratory arrest, they were resuscitated. The animals in this report were not resuscitated. Thus sporadic breathing before unsuccessful recovery could result in the increased RMSSD and SDSD values in Block 3 of US rats. This increase also contributes to the increased NSD in that same block.

This study in time domain analyses for HRV may indicate that NSD of IBI may be the most useful in predicting fatal hypothermia when compared to other time domain analyses such as RMSSD and SDSD.

CONCLUSIONS

Future and ongoing studies including time domain and frequency domain analyses of hypothermic and hyperthermić rats can be used to further examine if signatures could be developed to detect and predict different kinds of physiological stress. Signatures developed from these studies could be tested on human ECG waveforms. Then algorithms developed for these signatures could be integrated into the Warfighter Physiological Status Monitor (WPSM) or used to forecast responses of IBI in the SCENARIO prediction model.

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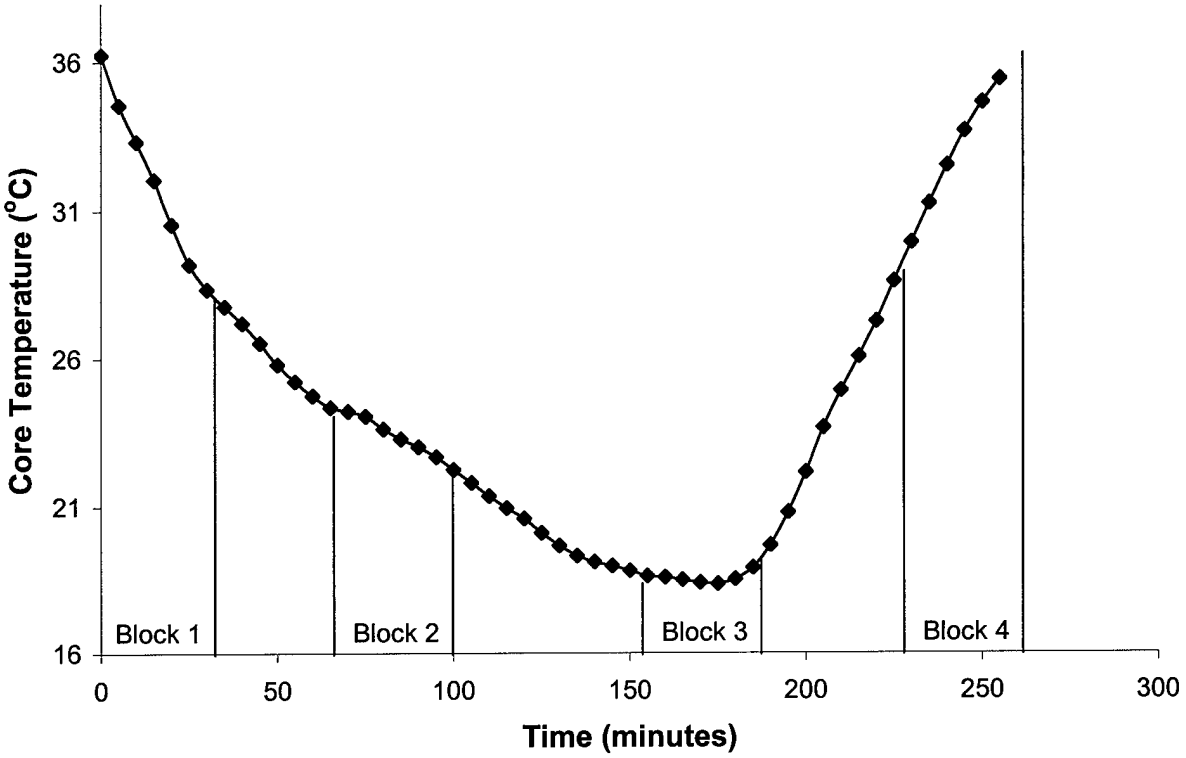
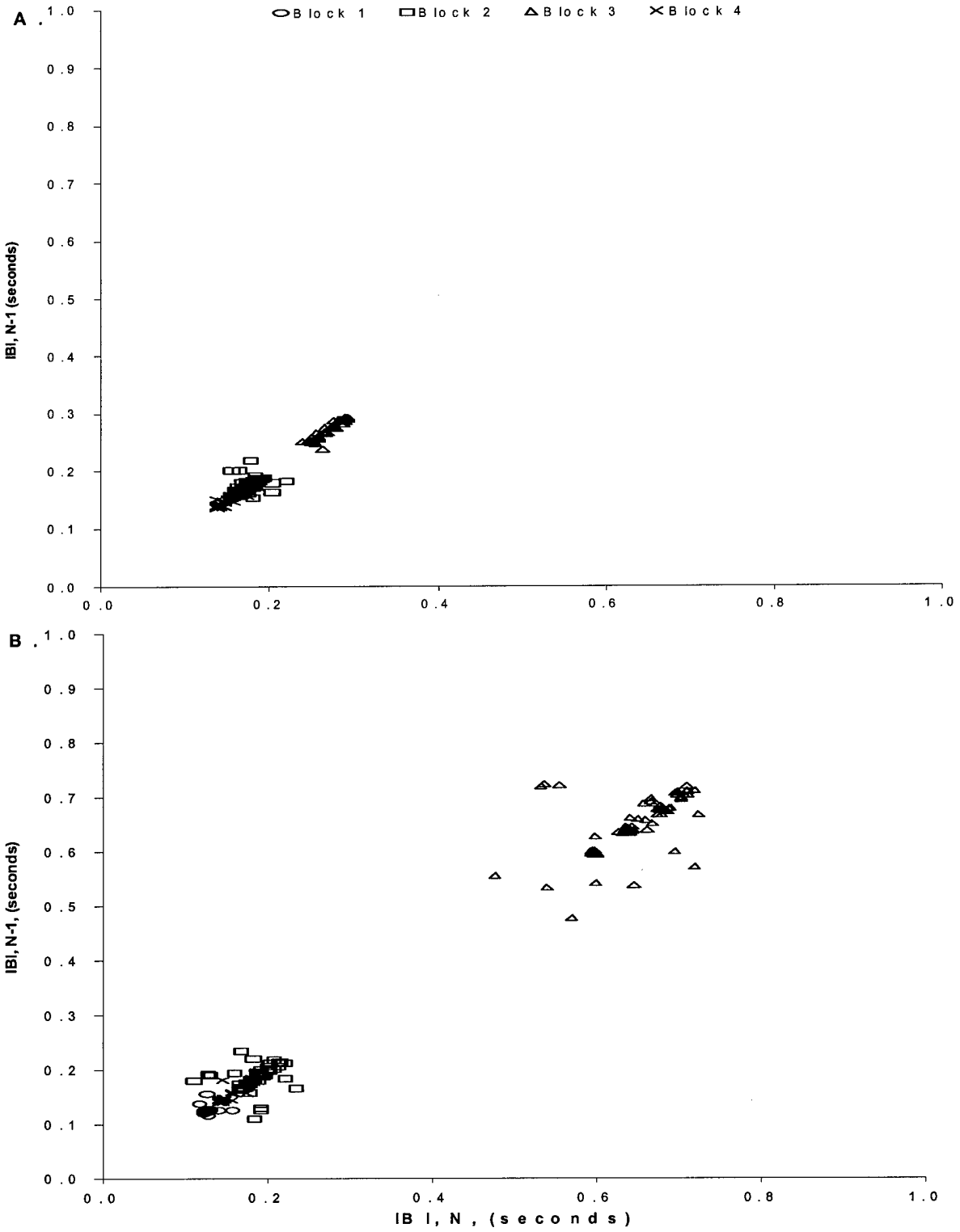
APPENDIX A: Figures 1-12

Figure 1. Representative Cooling and Re-warming Curve



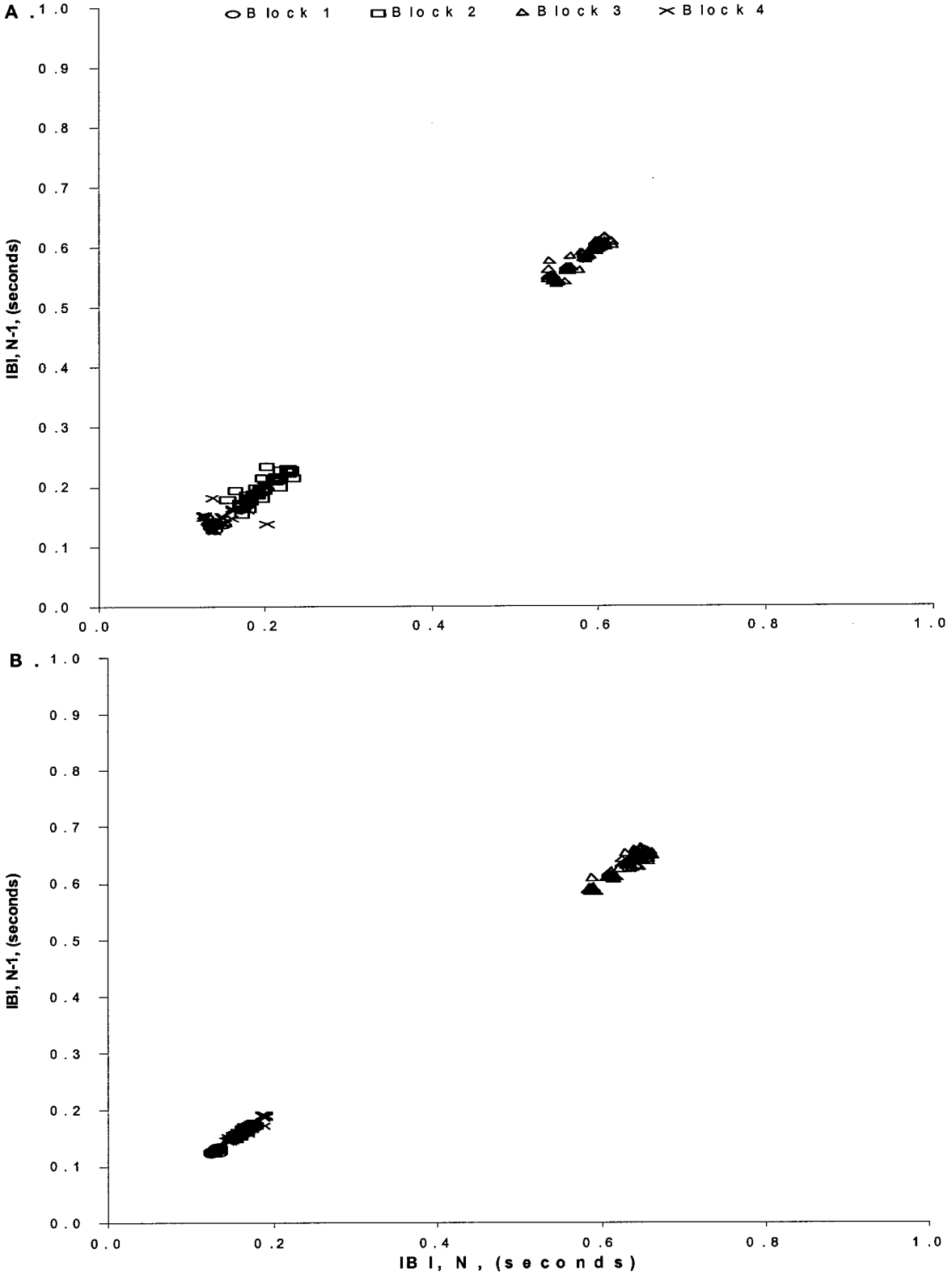
This figure is a representative cooling and rewarming curve used to designate the IBI data used for each block. Block 1 represents Tc = 34.0 ± 3.2 °C, Block 2 Tc = 24.8 ± 2.1 °C, Block 3 Tc = 19.8 ± 0.7 °C and Block 4 32.7 ± 1.8 °C.

Figure 2. Lorenz plot for Rat 98293 Days 1 and 12



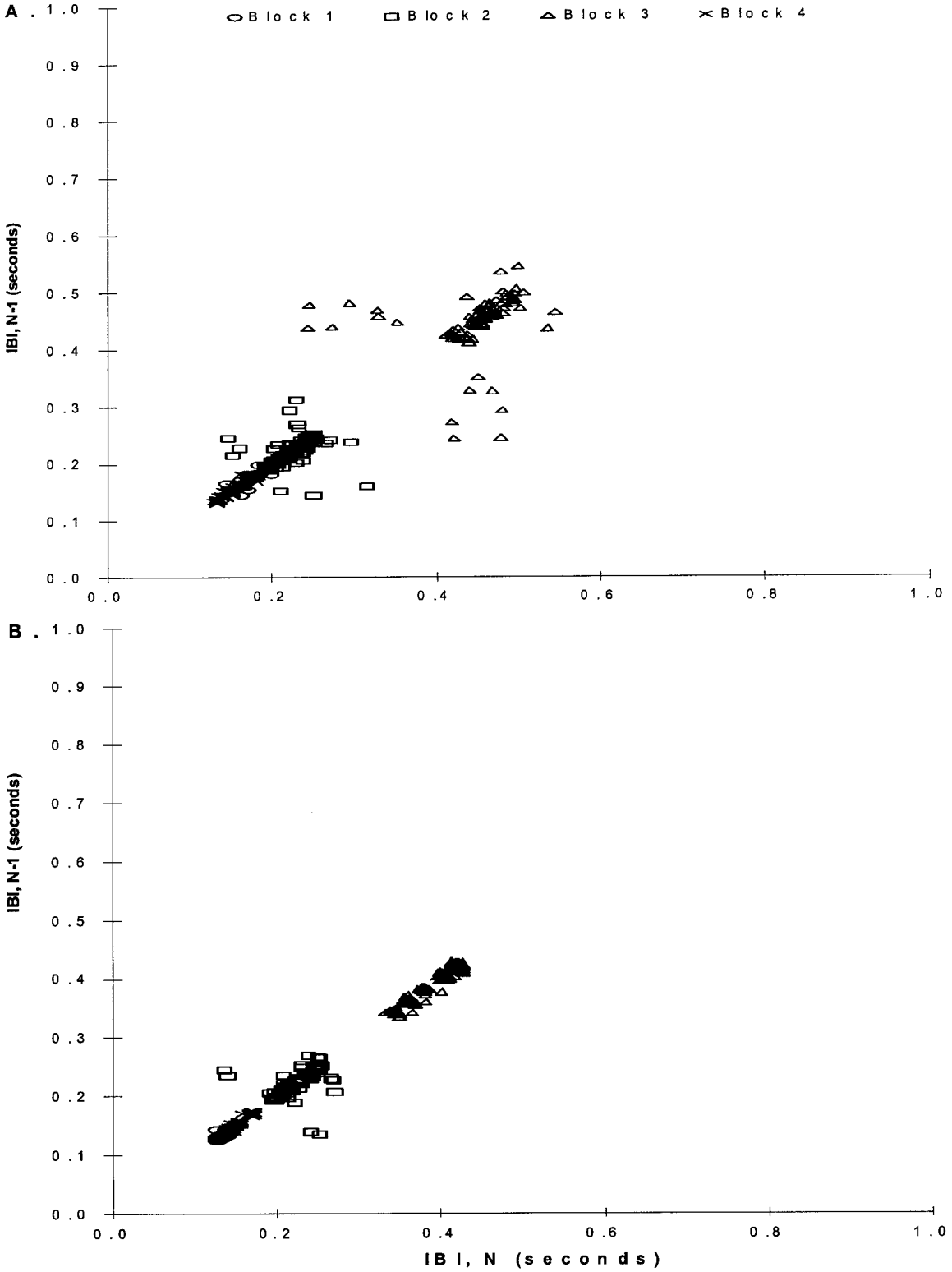
Figures 2A. and 2B.demonstrate the distribution of IBI's during cooling and re-warming of Rat 98293 during the first and second trials. The second trial was performed 12 days after the first trial. During both trials, the animal was successfully cooled and re-warmed.

Figure 3. Lorenz plots for Rat 98296 Days 1 and 29



Figures 3A. and 3B.demonstrates the distribution of IBI's during cooling and rewarming of Rat 98296 during the first and seconds trials. The second trial was performed 29 days after the first trial. During both trials, the animal was successfully cooled and rewarmed.

Figure 4. Lorenz plots for Rat 98297 Days 1 and 4



Figures 4A. and 4B. demonstrate the distribution of IBI's during cooling and re-warming of Rat 98297 during the first and second trials. The second trial was preformed 4 days after the first trial. During both trials, the animal was successfully cooled and re-warmed.

Figure 5. Lorenz plots for Rat 98300 Days 1 and 6

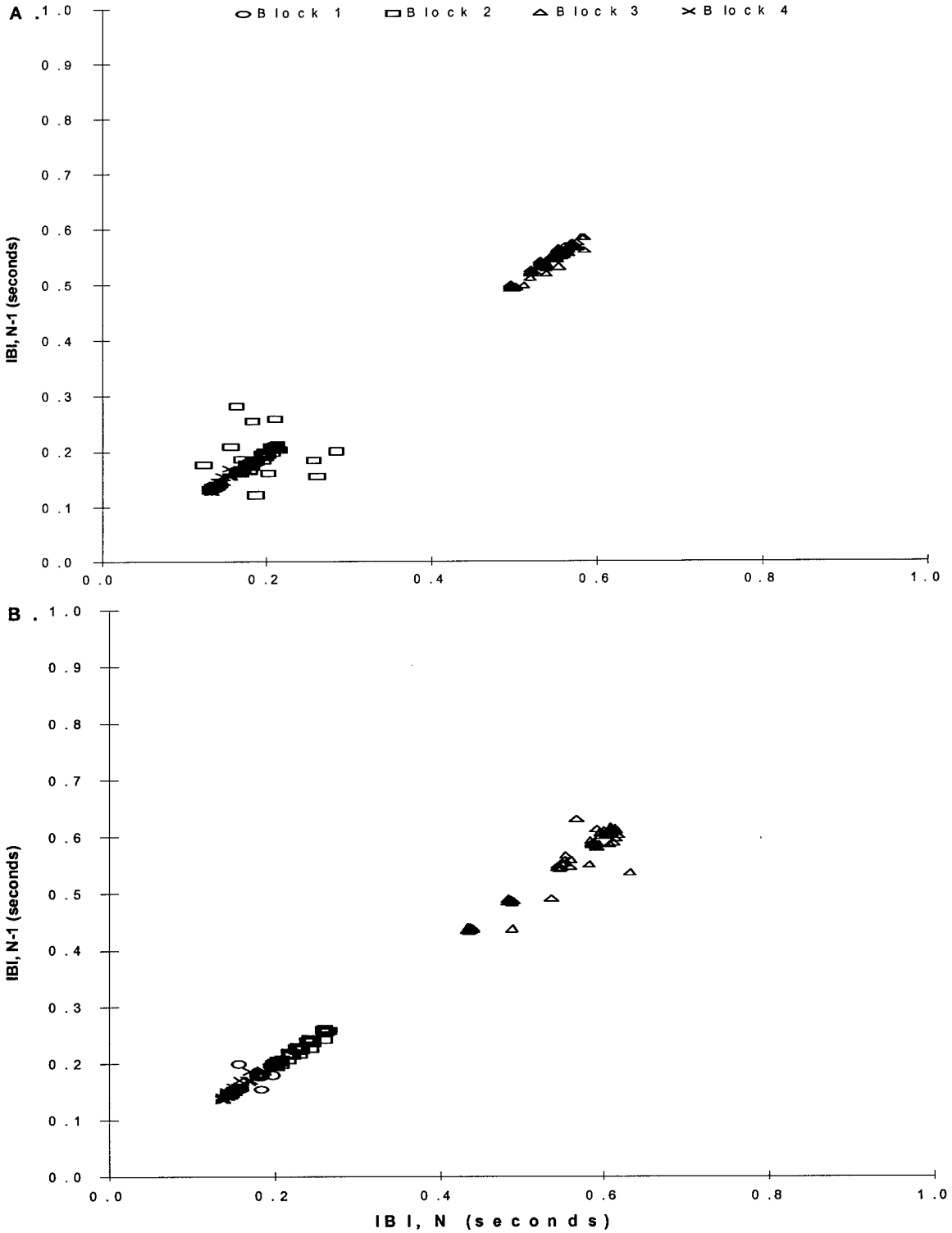


Figure 5A. and 5B demonstrate the distribution of IBI's during cooling and re-warming of Rat 98300 during the first and second trials. The second trial was performed 6 days after the first trial. During both trials, the animal was successfully cooled and re-warmed.

Figure 6. Lorenz plots for Rat 00244 and 00246

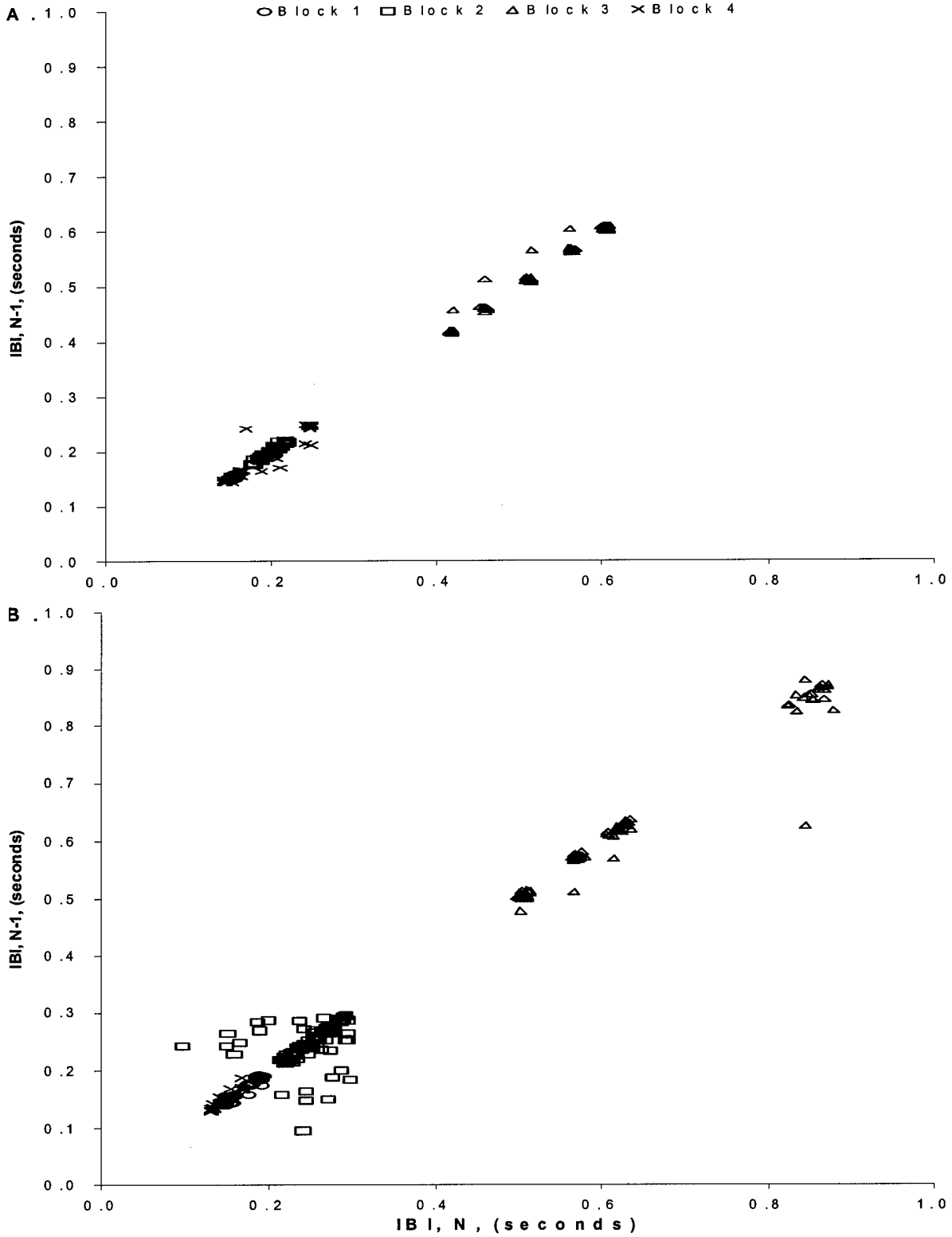


Figure 6A. and 6B. demonstrate the distribution of IBI's during cooling and re-warming of Rats 00244 and 00246, respectively. These animals were successfully cooled and re-warmed.

Figure 7. Lorenz plots for Rat 00248 and 00250

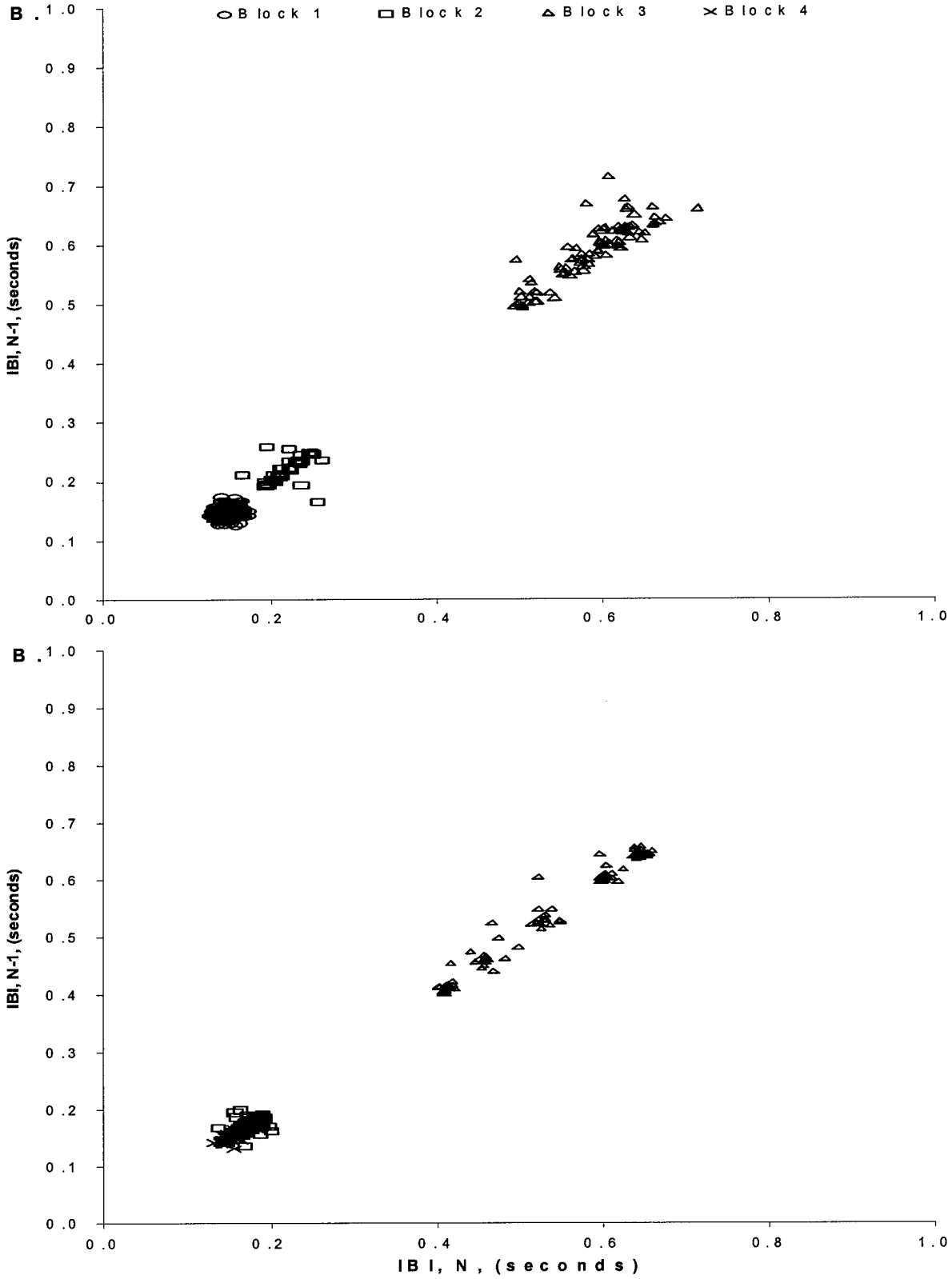


Figure 7A. and 7B. demonstrates the distribution of IBI's during cooling and re-warming of Rats 00248 and 00250, respectively. These animals were successfully cooled and re-warmed.

Figure 8. Lorenz plots for Rats 00251 and 99063

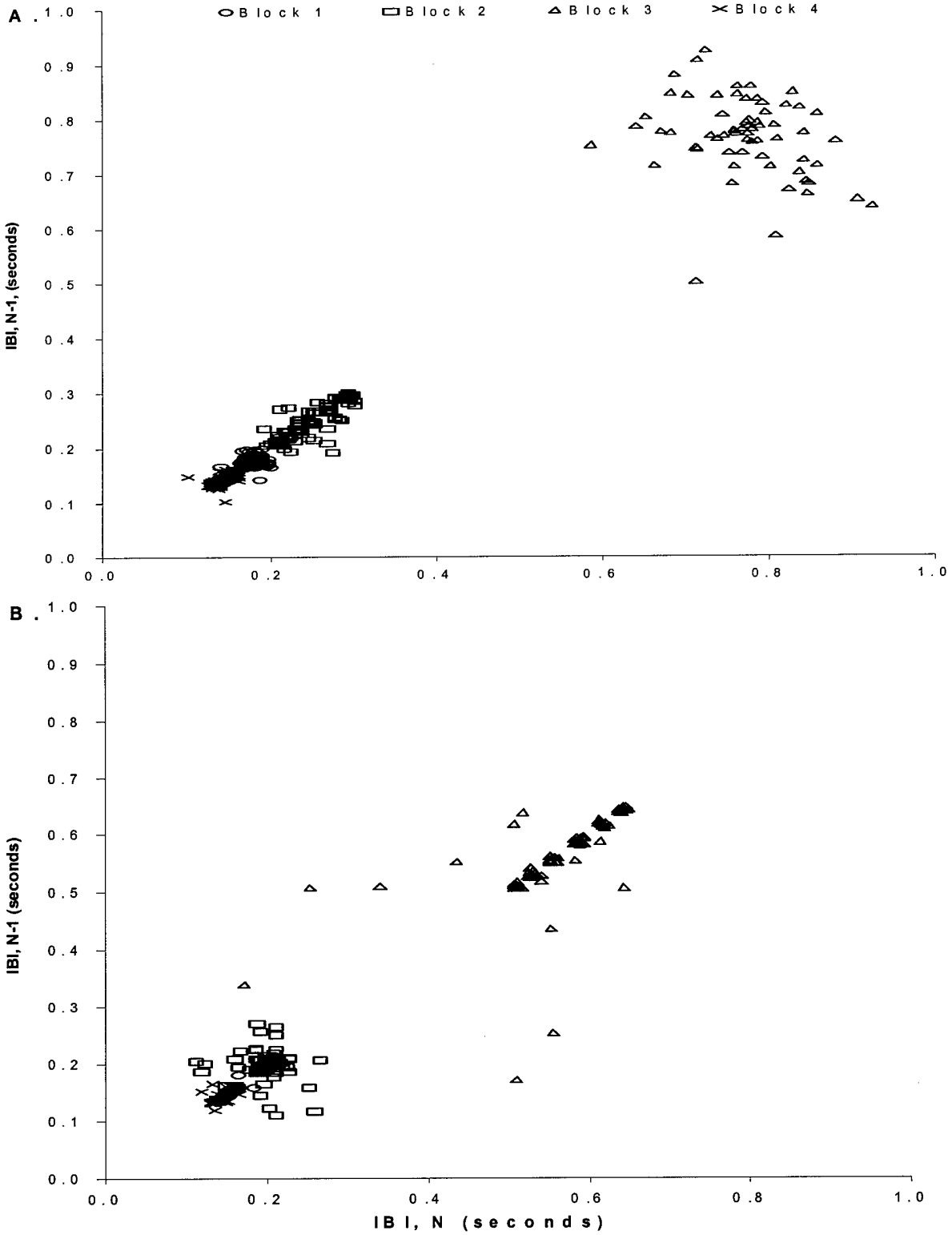


Figure 8A. and 8B. demonstrates the distribution of IBI's during cooling and re-warming of Rats 00251 and 99063, respectively. These animals were successfully cooled and re-warmed.

Figure 9. Lorenz plots for Rats 98298 and 99063

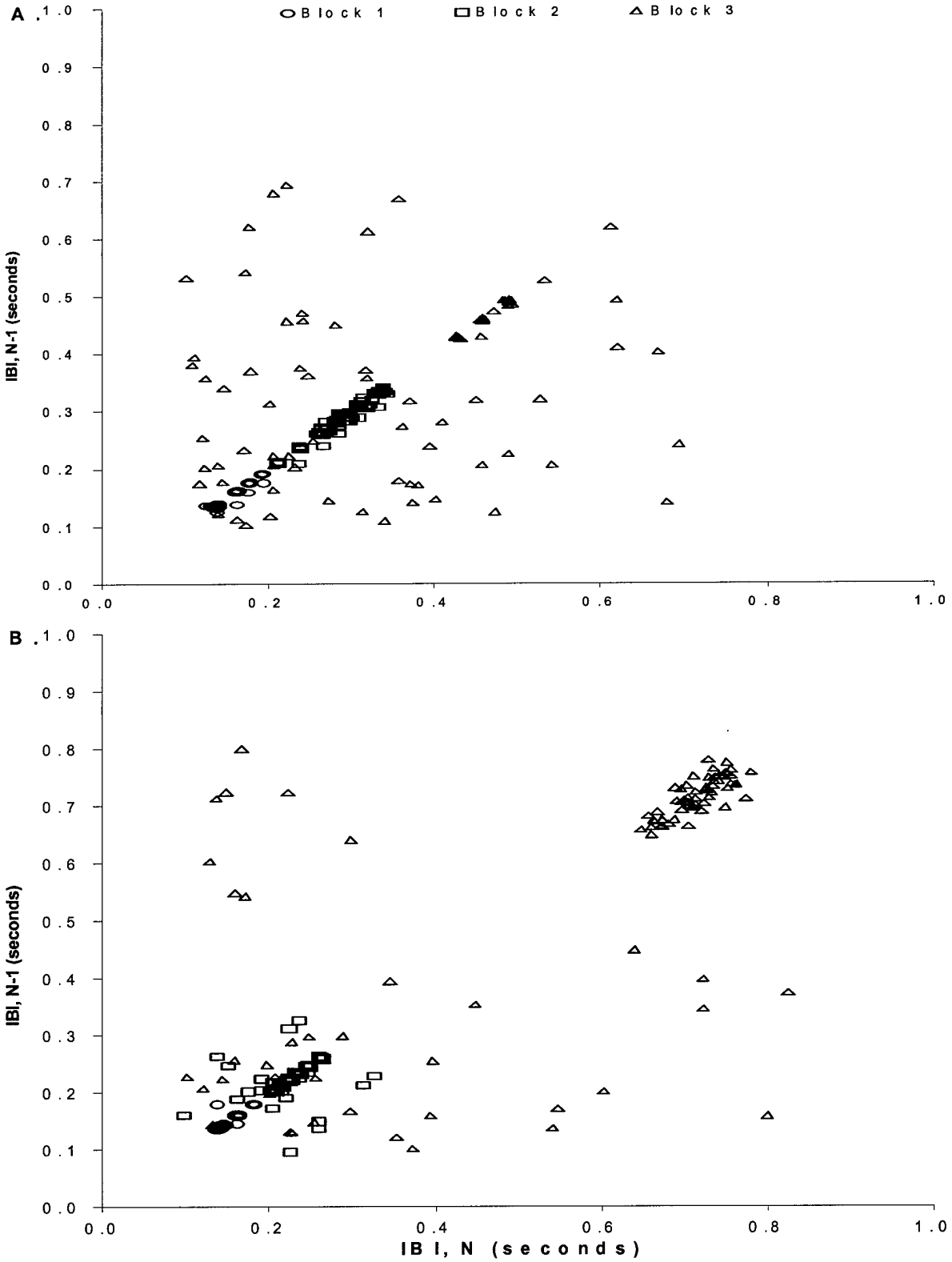


Figure 9A. and 9B. demonstrate the distribution of IBI's during cooling and re-warming of Rats 98293 and 99063, respectively. The second trial for 10B was performed 6 days after the first trial. These animals were unsuccessfully cooled and re-warmed.

Figure 10. Lorenz plots for 00241 and 00242

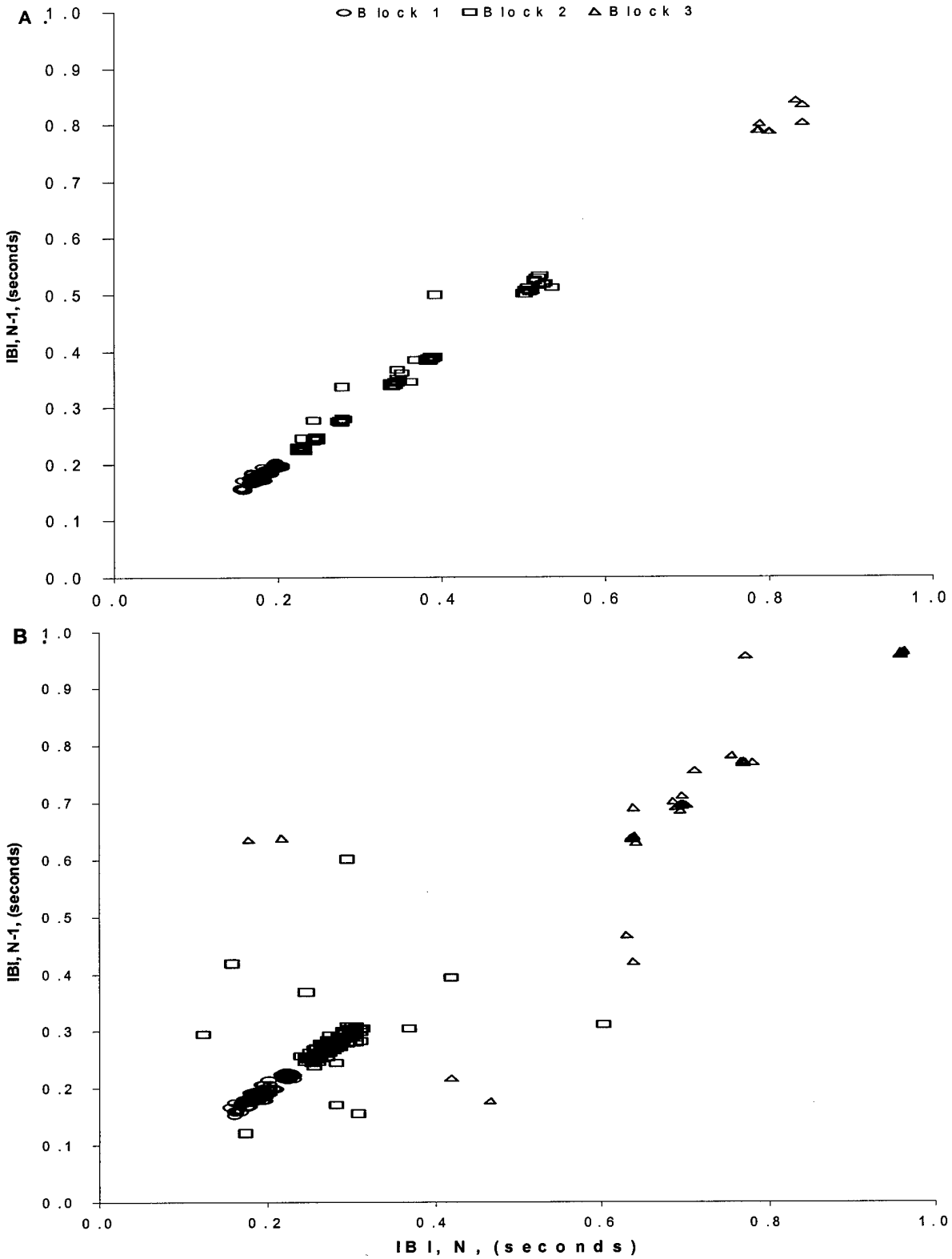


Figure 10A. and 10B. demonstrates the distribution of IBI's during cooling and re-warming for Rats 00241 and 00242, respectively. These animals were unsuccessfully cooled and re-warmed.

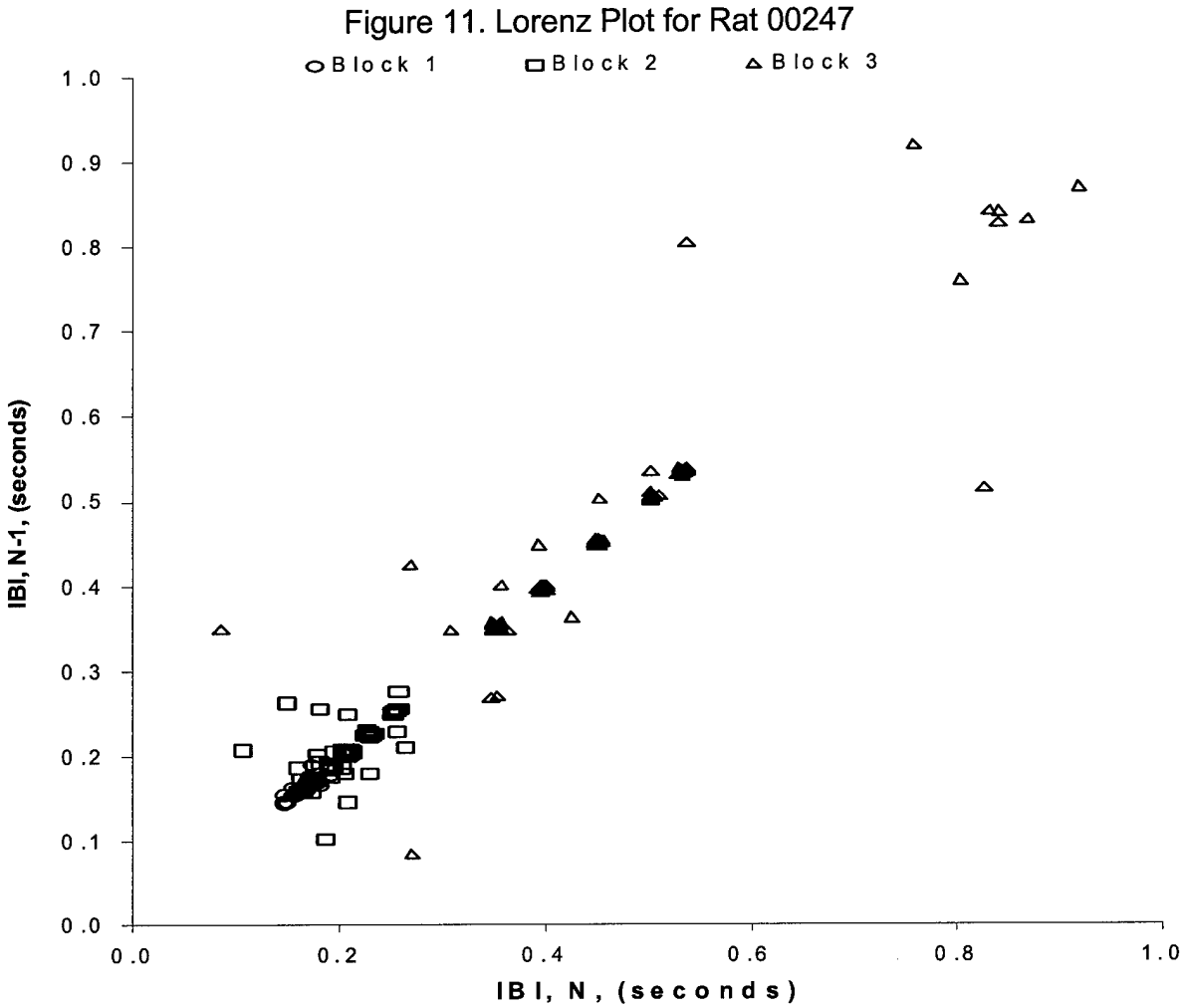


Figure 11. demonstrates the distribution of IBI's during cooling and re-warming for Rat 00247. This animal was unsuccessfully cooled and re-warmed.

Figure 12. Mean Inter-beat Intervals and Normalized Standard Deviations for Rats Successfully and Unsuccessfully cooled and rewarmed

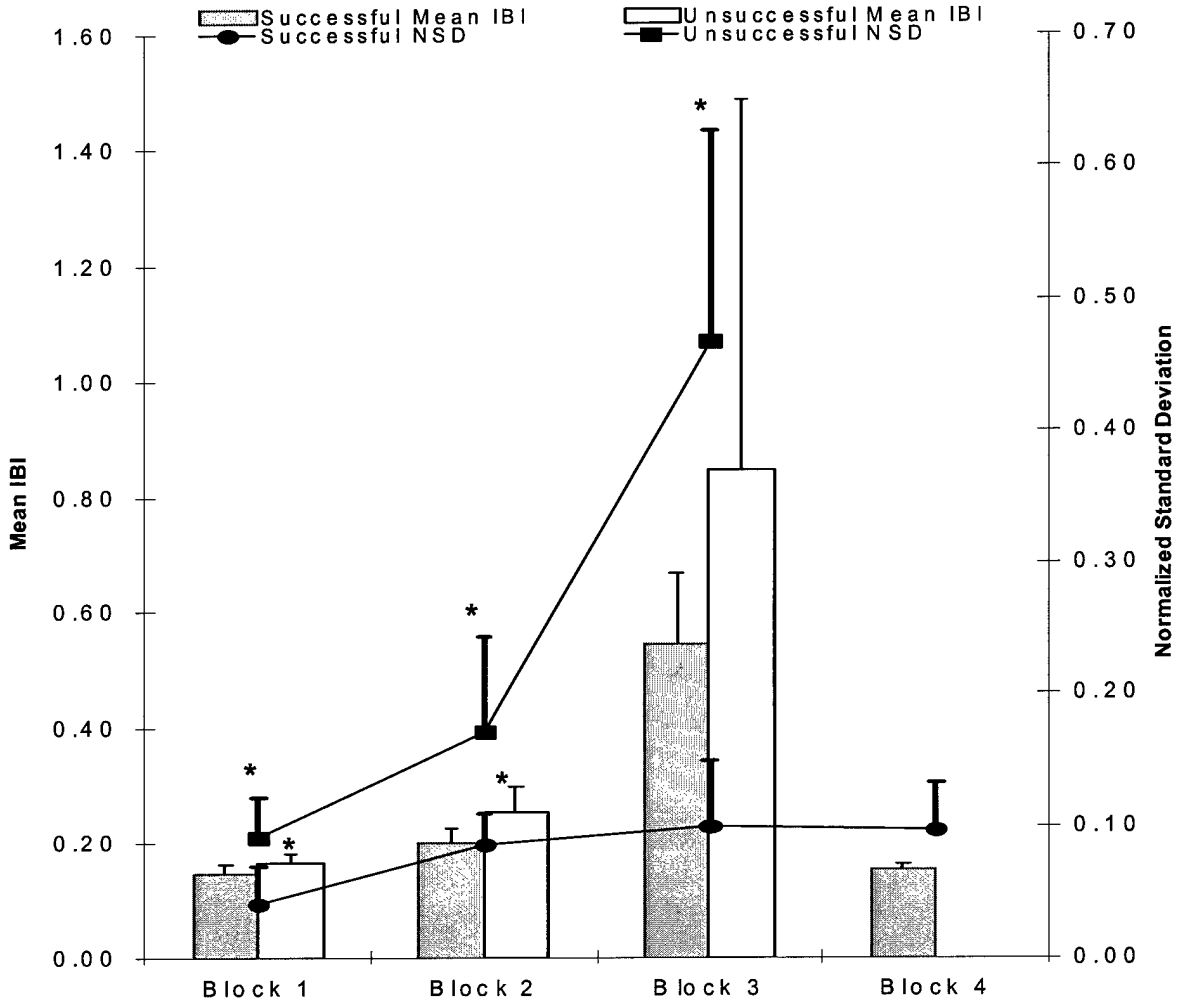


Figure 12. demonstrates the mean IBI's and NSD's during the indicated core temperatures of successfully and unsuccessfully cooled and rewarmed rats (mean + std). Bars represent the IBI's and Lines represent NSD's. The Tc's of the S rats are as follows for Blocks 1-4: 34.2 ± 3.1, 24.9 ± 2.1, 19.8 ± 0.6, and 32.7 ± 1.8°C. For US rats the Tc's for Blocks 1-3 are as follows: 34.5 ± 2.8, 24.6 ± 2.2, and 19.8 ± 0.7°C. The symbol " * " indicates a significant difference from S rats.

APPENDIX B: TABLE 1

Table 1. Time domain measures of HRV in S and US cooled and re-warmed rats

	Mean IBI		NSD		RMSSD		SDSD	
Block	**S**	**US**	**S**	**US**	**S**	**US**	**S**	**US**
1	.1471 ± .0040	.1650 ± .0069 φ	.0408 ± .0077	.0915 ± .0139 φ	.0029 ± .0006	.0048 ± .0022	.0029 ± .0006	.0032 ± .0007
2	.2018 ± .0067	.2537 ± .0196 φ	.0864 ± .0062	.1713 ± .0324 φ	.0112 ± .0022	.0196 ± .0071	.0098 ± .0017	.0197 ± .0072
3	.5448 ± .0324	.8474 ± .2857	.0999 ± .0133	.4676 ± .0708 φ	.0231 ± .0059	.3483 ± .1509 φ	.0233 ± .0060	.3403 ± .1534 φ
4	.1525 ± .0026	--	.0978± .0095	--	.0031 ± .0004	--	.0032 ± .0004	--

This table demonstrates the mean ± standard error of time domain measures of HRV in S and US cooled and re-warmed rats. The symbol "φ" indicates significant differences from S rats ($p<0.05$).

DISCLAIMER

The views, opinions, and/or findings contained in this report are those of the authors and should not be construed as official Department of the Army position, policy or decision, unless so designated by other official documentation. In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals, Department of Health and Human Services, revised 1996. The United States Army Research Institute of Environmental Medicine is an AAALAC-I accredited facility and will continue to adhere to the standards and requirements thereof. Citations of commercial organizations and trade names do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.